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LOCOMOTION AND PROPAGATED SKIN IMPULSES IN SALPS (TUNICATA: THALIACEA)

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With the notable exception of the work of M. Fedele, whose major papers on salps in the period 1923–1933 are cited below, few serious attempts have been made to analyze the behavior of salps. Yet, being large transparent animals with clearly visible and well-defined muscle bands and nerves, with compact brains and interesting behavior, they and the doliolids are potentially the most promising of the tunicates for neurophysiological investigations. Because of their scarcity in most coastal regions, salps have been little studied. The present report is the first electrophysiological investigation of salp behavior and is correspondingly incomplete or tentative in many respects. The mechanism of locomotion has been studied using techniques similar to those adopted in previous studies of tunicate behavior. These include investigations of the locomotory control mechanisms in the larvacean *Oikopleura* (Bone and Mackie, 1975) and in the tadpole larva of an ascidian, *Dendrodoa* (Mackie and Bone, 1976). In both these cases, and now in salps, evidence has been obtained for the existence of "neuroid" conducting epithelia (Mackie, 1970) and for their role as sensory pathways in responses involving changes in locomotory activity.

Materials and Methods

The salps used in this study were caught in plankton nets or scooped from the water in glass bottles in the bay of Villefranche-sur-Mer, France, during the period December 1973 to March 1974. They were kept in circulating seawater aquaria at the Station Zoologique and were used for experiments soon after capture.

Taxonomic and biological accounts of the species used (*Thalia democratica, Salpa fusiformis, Salpa maxima, Ihlea punctata,* and *Pegea confoederata*) are given by Branconnot (1973). Ihle (1933, 1958) provides indispensible background information on salp morphology and relationships.

Formalin-fixed and fresh tissues were examined by Nomarski and phase contrast microscopy. Material was fixed for electron microscopy in 5% cacodylate-buffered glutaraldehyde in sea water, followed by postfixation in 1% osmium tetroxide. Sections were stained with uranyl acetate and lead citrate prior to examination with a Philips EM-200.

Fine polyethylene suction electrodes were used for recording externally from the muscle bands and conducting epithelia. Signals were amplified and displayed on a Grass Polygraph and simultaneously on a Tektronix storage oscilloscope. A Grass stimulator was used for electrical stimulation. Intracellular recordings were made with glass micropipettes filled with 3 M KCl having resistances in the range

30–50 megohms, in conjunction with a Medistor A35 electrometer amplifier and an FET amplifier having current injection bridge circuitry.

Results

Swimming patterns and muscle physiology

Locomotion in salps is brought about by rhythmic contractions of the muscle bands which lie in the body wall, acting in conjunction with the muscles of the inhalent and exhalent valves. Locomotion is normally in the forward direction, water being expelled through the posterior, exhalent valve. In the lab, recently collected salps swim most of the time but show quiescent interludes. In nature they probably swim nearly all the time and must do so in order to feed (Madin, 1974). There is no evidence that salps can control the angle at which they swim or that locomotion is directional, in the sense that they show behavioral taxes. However Fedele (1923, 1933b) showed that it was possible to alter the swimming pattern by appropriate stimulation. Stimulation of the hinder regions and exhalent valves causes accelerated forward swimming. Stimulation of the front region and inhalent valves causes a defensive response, which consists of an alteration of the normal sequence of muscle contractions: the exhalent valve shuts first (instead of the inhalent), followed by contraction of the body wall muscles. Thus, water is expelled from the inhalent valve. A similar response is seen when the inside of the pharynx is stimulated (Fedele, 1933a, c). These movements would presumably serve to prevent unsuitable objects from entering or becoming lodged in the pharynx, as does the squirting of a sessile ascidian, but they also automatically interrupt locomotion. Fedele (1923) states that these defensive movements can be elicited by photic, thermal, chemical or tactile stimuli.

Mature blastozooids of *S. fusiformis* exhibit a swimming rhythm of 0.5–0.8 beats per second. Suction electrodes attached to the muscle bands show that each contraction is correlated with a burst of muscle potentials (Fig. 1A). Resolution of these potentials, which rarely exceed 200 μV, falls off as the electrode is moved away from the muscle band, but an electromyogram can be picked up from any part of the animal even, in small specimens, from the surface of the test.

In forward locomotion the muscles which operate the inhalent valve fire about 40 msec before the main muscle bands and the muscles of the exhalent valve (Fig. 1B). Recordings from different muscle bands in the body wall show close synchrony in the onset and conclusion of contraction bursts both during forward (Fig. 1C) and reverse (Fig. 1D) locomotion, and it is evident that the main musculature fires more or less as a unit.

Gentle tactile stimulation of the inhalent valve (on its inner surface in Fig. 2A) causes a break in the rhythm of forward swimming, while stimulation of the exhalent valve causes accelerated forward swimming (Fig. 2B). Fedele's defensive response is exhibited in varying degrees according to the strength of stimulation. With weak stimulation, it may be a barely preceptible hesitation in the rhythm. With strong stimulation, prolonged inhibition or reverse locomotion takes place. These responses are accompanied by conducted epithelial potentials (skin pulses) which will be described below.

The reverse beat is a more powerful contraction than the forward and con-

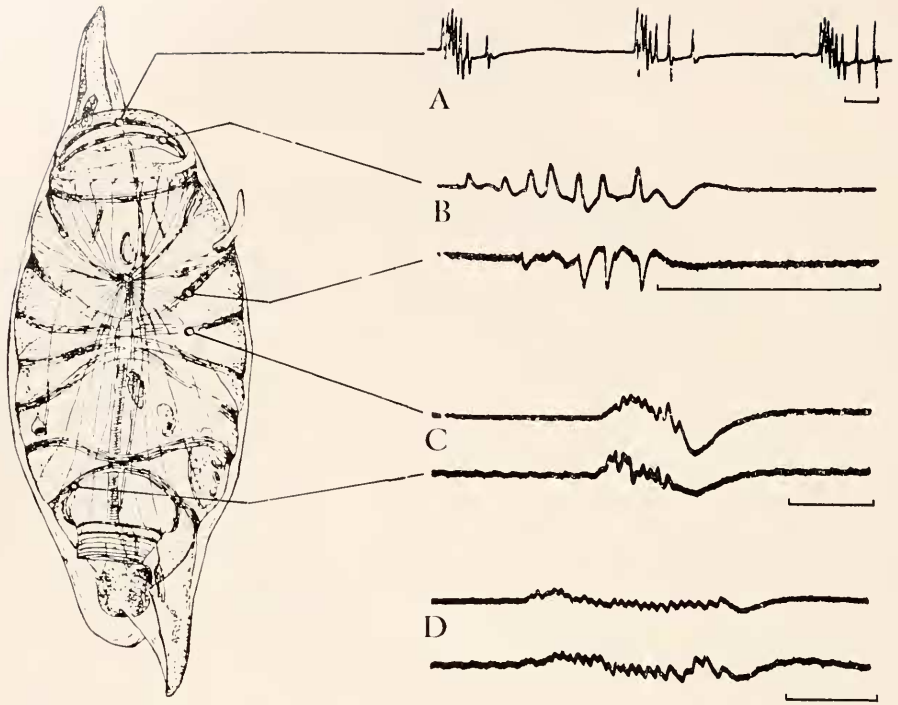

FIGURE 1. *Salpa fusiformis*, dorsal view of a blastozooid (from Fedele, 1933b) with extracellular recordings from the muscle bands (scales are 100 msec): A, forward swimming, series of contraction bursts from a muscle of the inhalent valve; B forward swimming, a single burst recorded simultaneously from the inhalent valve and from body wall muscle band I; C, forward swimming, a burst recorded from right band II and left band VI; and D as for C, but reverse swimming response.

sists of a longer burst of potentials (Fig. 1D). The beat frequency is also lower. The reversal of the firing sequence is illustrated in Figure 3. After a period of reverse locomotion, forward swimming is resumed spontaneously, sometimes after a very brief delay, sometimes after a longer interval. A residual inhibitory effect is

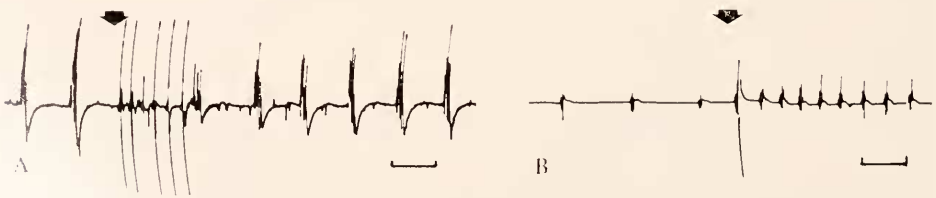

FIGURE 2. Alteration of the swimming rhythm in *S. fusiformis* (scales are 1 sec): A, blastozooid—a stimulus (arrow) elicits a burst of five anterior skin pulses and also causes a brief interruption of swimming, as seen in the retardation of swimming bursts and their attenuation; and B, young oozoid—a stimulus at the rear end (arrow) evokes a single outer skin pulse and accelerated swimming.

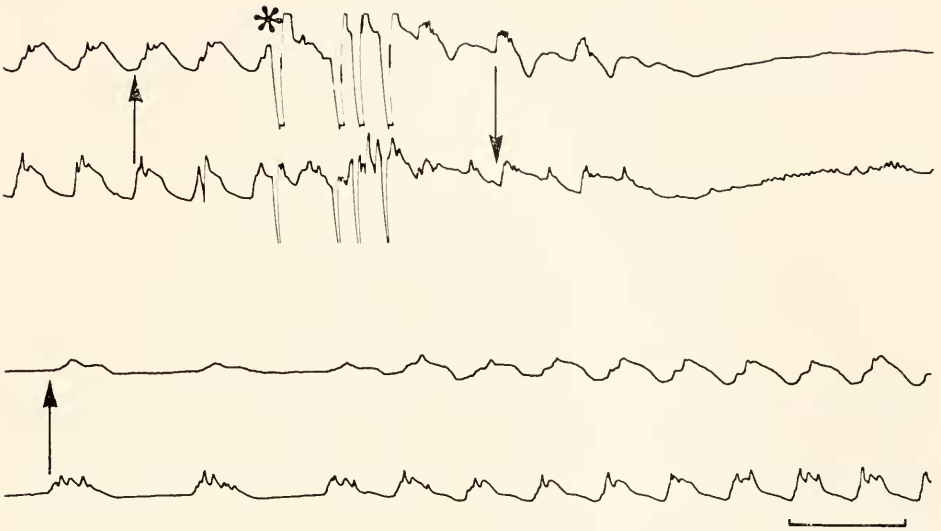

FIGURE 3. *S. fusiformis:* reversal of swimming. In each pair of records, the upper electrode was attached to the exhalent valve, the lower to the inhalent. Tactile stimulation of the inhalent valve (at asterisk) evoked a series of four outer skin pulses and the direction of swimming changed from forward (lower channel leading, arrow up) to reverse (arrow down). Reverse swimming then gave place to a period of complete inhibition of swimming. Eight seconds are omitted between the two pairs of records during which slow forward swimming (arrow up) was established, subsequently giving way abruptly to fast forward swimming. (Scale is 1 sec).

evident in the weakness of the first forward swimming beats after a period of quiescence, and the frequency may be abnormally low during this phase. This slow, "inhibited" forward swimming may change gradually to the normal pattern, but sometimes an abrupt change of pace is seen (Fig. 3).

The locomotory rhythm is markedly affected by changes in light intensity. Salps which are swimming rhythmically accelerate following a reduction in light intensity, and those which are quiescent can often be induced to start. An increase in intensity slows the rhythm or causes locomotory arrest. The significance of these reactions in the natural behavior of salps in the sea is uncertain. It is unlikely that vertical distribution is influenced by diurnal changes in light intensity (C. Apstein, cited by Ihle, 1958). The light OFF response might be a predator avoidance reaction ("shadow reflex") of the type described in some hydromedusae (Yoshida and Ohtsu, 1973).

The muscle bands in *S. fusiformis* are composed of parallel elongated fibers, flattened in cross section and showing cross striations. They differ little from those of other salps such as *Iasis* and *Thalia*, whose ultrastructure and innervation have been described recently (Bone and Ryan, 1973). There are several neuromuscular junctions on each fiber, derived from axons passing out from the central nervous system in different nerve bundles. A single axon may innervate several muscle fibers; as Fedele (1925) showed, endplates may be intercalary.

To prevent swimming, it is only necessary to sever the nerves between the

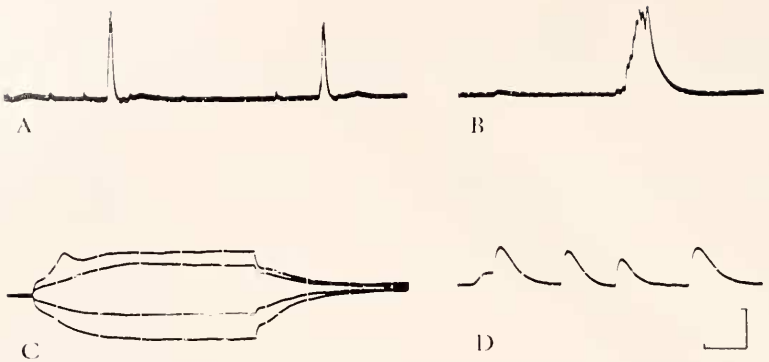

FIGURE 4. Intracellular records from salp muscle bands (A-C, *S. fusiformis;* D, *Ihlea punctata*): A, contraction bursts recorded in an intact, swimming animal (scales are 500 msec, 10 mV); B, a burst from the same preparation on expanded time scale (100 msec, 10 mV); C, denervated fiber injected with hyper- and depolarizing current pulses of 2×10^{-8} and 4×10^{-8} A. Note rectification of the response to the stronger depolarizing current pulse (scale is 20 msec, 20 mV); and D, denervated, fibrillating fiber showing junctional potentials recorded near a neuromuscular junction (scale 50 msec, 10 mV).

brain and the muscles. This was done in experiments which required placement of two microelectrodes in the same fiber simultaneously. The muscles can be made to contract by stimulating their motor nerves, but the nerves are too small for this to be done conveniently.

Intracellular recordings from muscle fibers in *Salpa fusiformis* and *Ihlea punctata* showed negative resting potentials in the range 50–55 mV. During spontaneous foward swimming, bursts of potentials summing to a maximum amplitude of 25 mV were recorded (Fig. 4A, B). These potentials correspond to the potentials recorded extracellularly, shown in Figure 1. The amplitude of contraction presumably depends on the duration of the burst and on the number and frequency relationships of the potentials, which will determine the level of depolarization, but this needs to be verified directly. During partial photic inhibition of swimming the amplitude of the summed potentials may drop by more than 50%, which visibily results in feeble contractions.

We regard these potentials as neuromuscular junctional potentials (JPs). Their numbers and frequency relationships probably reflect the pattern of motor nerve impulses arriving at the junction. Recordings from presumed motor neurons in the isolated brain show similar patterns of impulses. Scattered JPs or small bursts of these potentials are sometimes seen in the intervals between the swimming contractions (Fig. 4A, B), but they cause no detectable shortening of the fiber. Denervated fibers are usually electrically silent at first, but start to show increased JP activity after a few minutes and eventually start to fibrillate. These changes are exacerbated by operations in the region of the muscle which involve opening up the haemocoel.

Salp muscle fibers show active membrane responses to depolarizing currents injected through a microelectrode (Fig. 4C). These responses are graded according to the amount of current passed. The largest responses induced had amplitudes of 20–25 mV. Responses have been seen with as little as 10 nAmp current.

Current pulses injected into one fiber induced no measurable voltage changes in the fibers on either side. This lack of lateral coupling is in accordance with the absence of gap junctions between adjacent fibers reported by Bone and Ryan (1973).

While it is hard to visualize neuromuscular junctions in an intact, swimming salp because of the movement and thickness of the preparation, it is not hard to see the junctions in denervated, flattened muscle preparations and to insert electrodes at known distances from neuromuscular junctions. Fibrillation potentials (Fig. 4D) recorded close to junctions appeared to be larger than the same events recorded further away; while this observation cannot yet be put on a precise quantitative basis, it serves as an indication that the junctions are still the sites of origin of the potentials recorded after denervation. Fedele (1933b) found that, in muscle bands partially isolated from the brain by nerve section, paralysis was restricted to denervated zones. This observation fits the general picture arrived at here, as it indicates that the muscle fibers are incapable of propagative electrogenesis.

There is good evidence for cholinergic transmission in tunicates (Florey, 1967), and salp neuromuscular junctions are characterized by vesicles resembling known cholinergic vesicles in other animals. The presumption of cholinergic transmission is supported by our observations on the effects of acetylcholine, curare and eserine on exposed muscle fibers; but the effects of these and other drugs need to be explored further and will be reported elsewhere.

Origin of the locomotor rhythm

Fedele (1933b) showed by surgical operations, and we have confirmed, that the swimming rhythm originates in the brain. Severing the nerves to the muscles causes paralysis. A few recordings were made from isolated brains removed from the animal and pinned out in sea water after dissecting away the covering epithelia. On a single occasion, a cell showing the characteristics of a pacemaker neuron was penetrated. This cell, from the brain of *Thalia democratica*, was impaled for 20 min, during which it fired regularly 1.5–1.7 times per second (Fig. 5A, B). Spike threshold lay at −55 mV. Following the spike, the cell hyperpolarized to −65 mV, then depolarized again to a new spike threshold. There was no sign of synaptic input and, although the cell could have been driven by another cell or cells, it was assumed to be endogenously rhythmic. Spike peak lay at −40 mV, which suggests that the electrode was in the cell soma, which was passively invaded by spikes initiated at some distance down the axon (*c.f.*, Hoyle and Burrows, 1973). Hyperpolarizing the cell slowed the rhythm and depolarizing it accelerated it (Fig. 5C, D). Folowing the current pulse, rebound excitation and inhibition effects were evident.

A second type of cell was identified in several separate recordings from brains of *Salpa fusiformis* (Fig. 5E, F). The electrode was situated in the superficial layer of the brain near the posterior end, the region in which large motoneuron somata are located (Fedele, 1933b). The low resting potential (−25 mV) and small size of the transient potentials are incompatible with a normal, stable intracellular placement. Presumably, these are proximity recordings or represent partial penetrations. The rhythm in this case (0.5/sec) lay within the normal

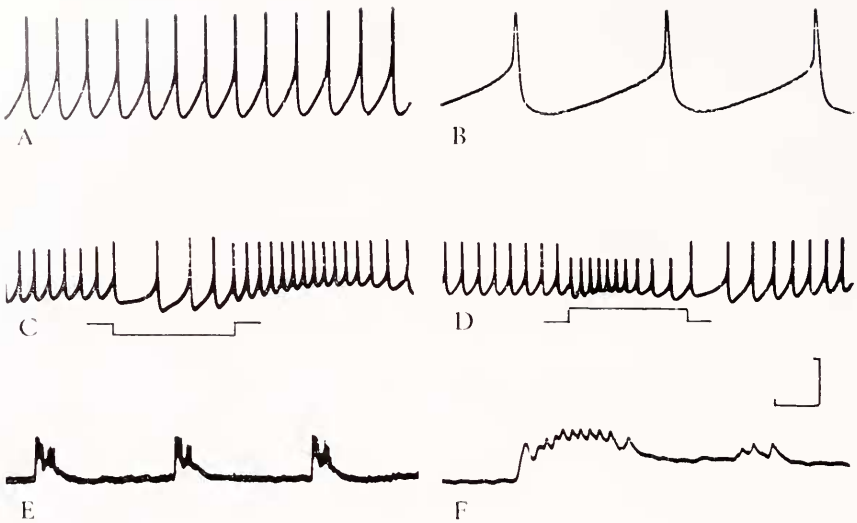

FIGURE 5. Recordings from isolated brains in *Thalia democratica* (A-D) and *Salpa fusiformis* (E, F). The former are from a pacemaker neuron and the latter from a presumed locomotory motoneuron, as explained further in the text. Scales are: A, 1 sec, 10 mV; B, 200 msec, 10 mV; C, D, 2 sec, 20 mV; E, 1 sec, 10 mV, and F, 50 msec, 10 mV.

range for the locomotory rhythm of this species and size of salp. As in the intact animal, the rhythm was affected by changes in light intensity (Fig. 6). This would be expected, since the brain incorporates ocelli. The pattern of events comprising the bursts resembles the pattern of JPs recorded from muscle cells both in number and frequency relationships. There can be little doubt that cells showing these impulse patterns are the motor neurons which control swimming.

The salp brain obviously merits further study. The circuitry is probably fairly simple. Fedele (1933b) found small "commissural" neurons in the outer layer (rind) which, he suggested, were responsible for synchronizing the activity of the mononeurons. He found that destruction of the central core of the ganglion failed to interfere with the rhythm, but that thereafter the rhythm was no longer modifiable by sensory input. Damage to the rind interfered directly with the

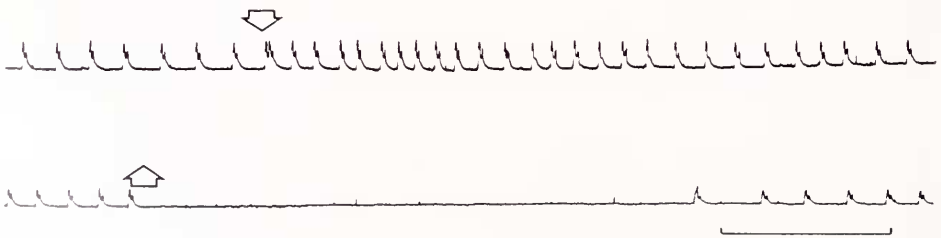

FIGURE 6. Microelectrode recording from presumed motor neuron in the brain of *S. fusiformis*. At light-OFF (arrow down) the rhythm is accelerated for about 10 seconds. At ON (arrow up) swimming is inhibited for 33 seconds. Scale is 10 sec.

rhythm. We also have found that a needle can be thrust repeatedly through the center of the brain without destroying the rhythm. It appears that the rhythm is generated by neurons with somata in the rind, while the core is the site of synaptic input from afferent neurons.

Skin pulse system

The term skin pulse (SP) refers to an impulse generated and conducted by epithelial cells without the intervention of nerves. However SPs can excite neurons. In *Oikopleura* excitation evoked by touching the skin is transmitted by skin cells as SPs, enters neurons and is conducted to the caudal ganglion, where it causes a change in the locomotory rhythm (Bone and Mackie, 1975). Conducting epithelia are well-developed in the Salpidae. The following account refers to *S. fusiformis*, where the excitable epithelia have been localized to three regions. The functional significance of this zonation is still unclear.

The main zone consists of the outer, ectodermal epithelium which underlies the test. Signals recorded from this epithelium (Figs. 2B, 8, 9, 11) are referred to as outer skin pulses (OSPs).

A second zone (in fact, a pair of similar zones) consists of the endodermal epithelium covering large areas of the pharynx and the walls of the gill bar. The area to the left of the endostyle including the left wall of the gill bar forms one self-contained conducting field, and the equivalent areas on the right form another (Figs. 7, 8). The pharyngeal wall is excitable only in its ventral and lower lateral regions, up to a line approximately level with the bottoms of the muscle bands. Sensitivity disappears rapidly dorsal to this line except in the gill bar, which is sensitive up to its dorsal attachment. Reference will be made to left and right inner skin pulses (LISPs, RISPs).

A third zone is the area of inner epithelium lying between the peripharyngeal bands and the border of the inhalent siphon (Fig. 7). Impulses recorded in this zone may be termed anterior skin pulses (ASPs), examples of which are shown in Figure 2A. The comparable area to the rear of the animal, posterior to the ventral attachment of the gill bar is not excitable.

Delineation of the excitable zones is complicated by the fact that skin impulses are recorded extracellularly as large events, often exceeding 1 mV and can consequently be picked up by electrodes quite far from the active site. Even LISPs and RISPs, which are relatively small events (less than 0.5 mV), can sometimes be picked up by a sensitive electrode in each other's territories (Fig. 8). The excitable epithelia enclose the haemocoel and electrotonic current flow through the blood is probably responsible for signal pickup in distant regions. OSPs can be recorded on the pharyngeal side, although their wave forms may be inverted or distorted here compared with recordings from the outer skin itself.

The location of the inner and anterior skin pulses in their respective zones has been verified by careful stimulation with fine glass needles. There is both indirect and direct evidence that OSPs are conducted by the less accessible outer epithelium. They are recorded at higher amplitudes and with fewer distortions from the outer epithelium than from the inner. They are conducted into the anterior and posterior prolongations of *S. fusiformis* blastozooids, where there is no inner epithelium. Direct proof of their location comes from microelectrode recordings from the outer

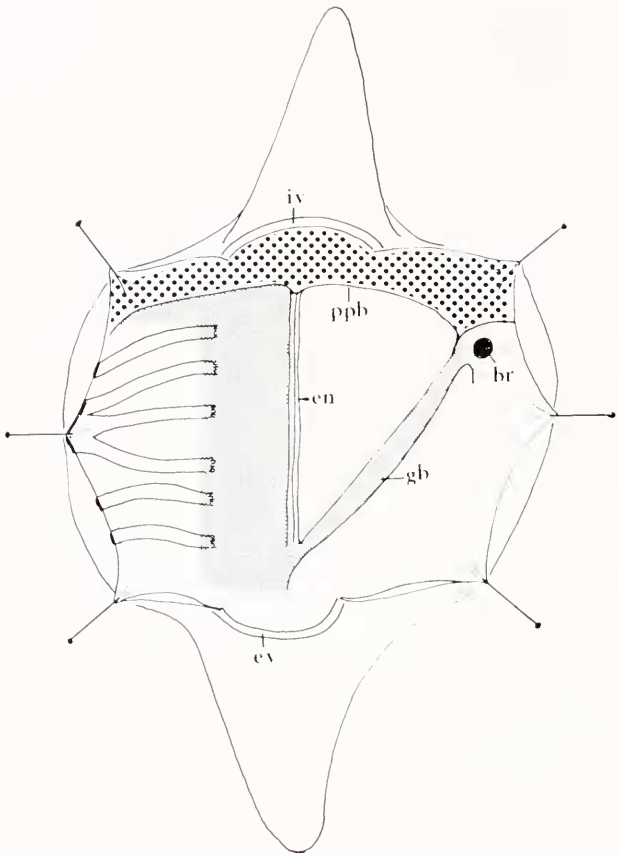

FIGURE 7. *S. fusiformis*, blastozooid: territories of anterior skin pulses (spots) and left inner skin pulses (stipple). The salp has been opened up from the dorsal side and pinned out with the gill bar to the right. Muscles are shown only on left. The right inner skin pulse zone is the mirror image of the left (br represents brain; en, endostyle; ev, exhalent valve; gb, gill bar; iv, inhalent valve; ppb, peripharyngeal band).

epithelium itself (Fig. 9). Here, a window was cut in the pharyngeal wall and a microelectrode was passed through it and inserted into the outer epithelium. Spikes recorded intracellularly from the epithelium correlated perfectly with events identified as OSPs on other grounds.

The epithelia are extremely thin. In electron micrographs of a young *S. fusiformis* blastozooid, the outer epithelium was found to be 0.5–1.4 μ thick, the inner 0.3–0.8 μ. The intracellular recordings were made from an older specimen which may have had thicker epithelia, but the tissues would still have been relatively thin, certainly less than 5 μ thick. Only three penetrations were made, all in the outer layer. The low resting potential (35 mV) and nonovershooting spike are indicative of cell injury. Penetration was only momentary. While these records serve to confirm the location of OSPs in the outer skin, the tissue is clearly

unsuitable for prolonged microelectrode work, and a thicker conducting epithelium must be sought if investigations are to be carried out on the electroionic characteristics of salp conducting epithelia. The ectoderm of the young stolon might be such a layer.

In conducting epithelia in amphibian tadpoles (Roberts, 1975) and hydrozoan coelenterates (Mackie, 1976) the cells are electrically coupled, and cell to cell transmission occurs by direct current flow, presumably through gap junctions, which have been located in the appropriate regions. A similar principle can be assumed to apply for tunicate conducting epithelia. Gap junctions (Fig. 9C) are seen connecting the cells of both inner and outer skin layers. The junctional region between adjacent cells is typically convoluted, and a *zonula occludens* occurs at the outer edge.

Brain removal and other operations which involve nervous or muscular lesions have no effect on the SP systems except insofar as they may damage, and so excite, the epithelia. The SP systems are not photosensitive. They are probably not spontaneously active but, being readily excited by damaging stimuli, are often exhibited in pinned preparations, where they may fire singly or in long bursts without apparent cause. In some cases, swimming movements, which would involve friction of the epithelia against pins or electrodes, appear to set off SP bursts. When a blastozooid undergoes autotomy from its neighbors in the chain, intensive OSP activity is recorded, probably due to damage of the outer epithelium at the adhesion plaques.

From the few, imperfect intracellular records obtained, OSPs are apparently conventional action potentials of approximately 10 msec duration (Fig. 9B), resembling epithelial spikes recorded in coelenterates (Mackie, 1976; Schwab, University of California, Irvine, personal communication) more closely than they do the action potentials recorded from larval amphibians (Roberts, 1975) and ascidian tadpoles (Mackie and Bone, 1976). Conduction velocity in the outer skin was measured at 17 cm/sec at 18° C in one preparation. In the same preparation ISPs were conducted along the gill at 8.5 cm/sec. The absolute refractory period for OSPs was measured at 7 msec in one example. OSPs can fire at up to 5/sec in the early stages of bursts.

Epithelial excitability is established early in embryonic development both in

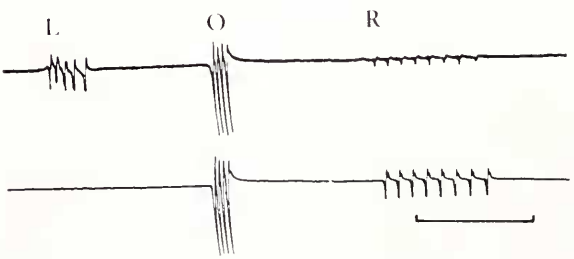

FIGURE 8. Comparison of OSPs, LISPs and RISPs recorded consecutively from the pharyngeal surface (*S. fusiformis*). The upper electrode was on the left of the endostyle, the lower on its right. Scratching the left side (at L) evoked LISPs, touching the right (R) evoked RISPs. OSPs were evoked by touching the outer surface of the salp, and are picked up electrotonically. Scale is 1 sec.

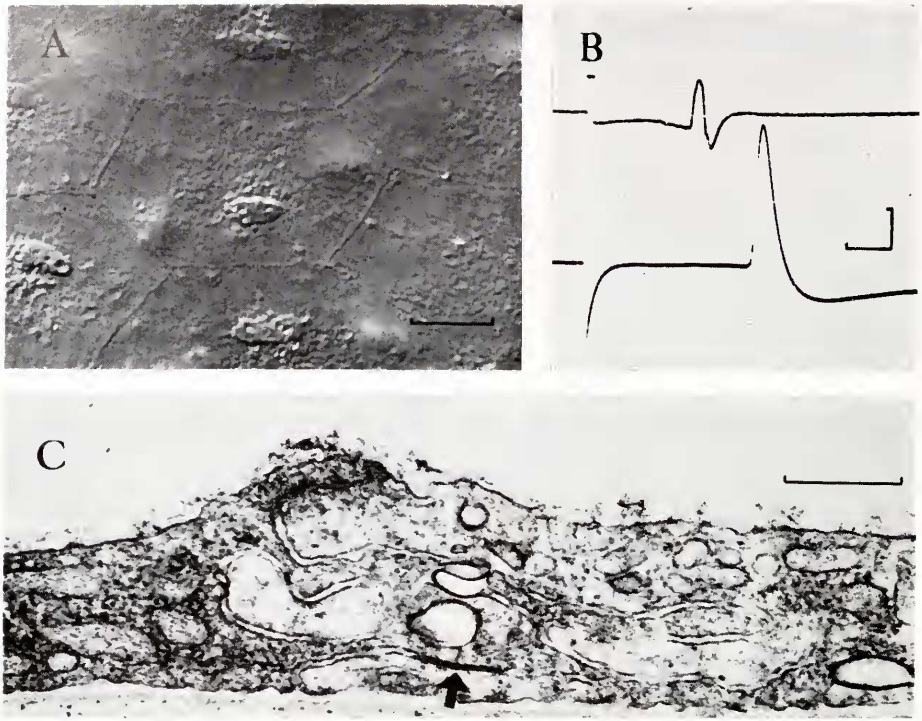

FIGURE 9. A shows epidermis of *S. fusiformis*, by Nomarski interference contrast microscopy) (scale is 20 μ). B shows a skin pulse recorded from this layer extracellularly (upper trace) and intracellularly (lower). The extracellular electrode was nearest to the stimulating site. The delay between the appearance of the signal on the two channels represents conduction time in the epithelium (scale is 10 msec, 500 μV, extracellular and 10 mV, intracellular). C shows a section through the outer epithelium in a junction region, showing a gap junction (arrow) (scale is 250 nm).

the case of oozoids and blastozooids. OSPs have been recorded in young oozoid embryos still attached to the parent, and in stolons in the process of subdivision into blastozooid chains. The question of SP communication in salp chains is discussed further below.

Skin pulses have no effect on the heartbeat, which is myogenic. There may be a delayed effect of ISPs on the beating of the gill bar cilia, but a nerve bundle runs through the bar and it is impossible to exclude the possibility that the effect on the cilia is due directly or indirectly to nervous excitation. Fedele (1933a) notes that food collection in salps involves coordination of ciliary activity in the gill bar and esophagus with mucous secretion by the endostyle. There is a food rejection reflex which involves muscular movements of the gut, cessation of mucous secretion and dissolution of the food web. Fedele attributes control of these effectors to the visceral nervous system, but the existence of the ISP system must now be taken into account.

The most obvious functional role for OSPs and ASPs lies in the regulation of locomotion. The sorts of tactile stimulation which cause acceleration, inhibition

and reversal of swimming almost always give rise to skin pulses. In Figures 2 and 3, for example, SPs are seen preceding or accompanying the change in swimming pattern, and appear to be causative of such changes. Epithelial pulses generated by touch are envisaged entering the nervous system and being relayed, as nerve impulses, to the brain. The most likely route is *via* the sensory nerve endings known to occur in the outer skin (Bone, 1959).

In a few cases an alteration was produced in swimming by delicate local stimulation which failed to elicit skin pulses. Here, direct stimulation of the sense organs may be presumed to have occurred.

If this interpretation is correct, the excitable epithelia would be functioning essentially as in *Oikopleura* (Bone and Mackie, 1975), extending the sensory field around ciliated neurosensory receptors lying within the epithelial sheets.

While it is difficult to elicit changes in locomotion without evoking skin pulses, a great deal of skin pulse activity can occur without any visible effect on locomotion. This is a common observation in pinned preparations where the stimulation due to the pins sets off frequent SP discharges. It appears likely that adaptation or fatigue occurs quickly at some step or steps along the sensory pathway. In nature, SPs are probably infrequent events which can affect swimming, but cease to do so on over-stimulation.

Coordination in salp chains

Fedele (1923) investigated the behavior of salp chains and found that, although the individual zooids have independent rhythms, all connected zooids show the same type of activity at any given time: in a resting chain, if one salp starts to swim, the others also start; if one stops, all stop; if one accelerates, so do most of the others; reverse swimming is propagated down the chain, for instance when the leading salp strikes an impassable object. For the chain, as for the individual, chemical, thermal, photic and tactile stimulation elicit these changes. If a chain is pinched in the middle, the two parts separate, the anterior half swimming forward at an accelerated speed, and the posterior going into reverse and swimming in the opposite direction.

We have little to add to Fedele's behavioral observations; so far as our own go, they confirm his. As Fedele observed, young chains are more responsive than older ones, and show reverse locomotion more readily. Some old chains will only swim forward. In chains of *Salpa maxima* whose zooids measured 1.5 cm in length no propagated responses of any kind were found, but in *S. fusiformis* and *P. confoederata* coordination was very clear. *S. fusiformis* chains swim equally well in either direction at velocities within the range 2.0–5.0 cm/sec, and all zooids swim in the same direction at any given time.

The question of how coordination is achieved has yet to be resolved. Ectodermal and endodermal continuity exists in stolons and young chains, but the endodermal connections are lost very early, and in some species the ectodermal connections become slender tubes (Fig. 10A) abutting on the adhesion plaques. There is no epithelial continuity across the plaque in older chains. Collections of ciliated sensory cells terminate at the plaques (Fedele, 1923; Bone, 1959), but nerves apparently do not cross directly from one zooid to the next. The flag organs (Bolles Lee, 1891) which are found only in aggregate salps and seem to

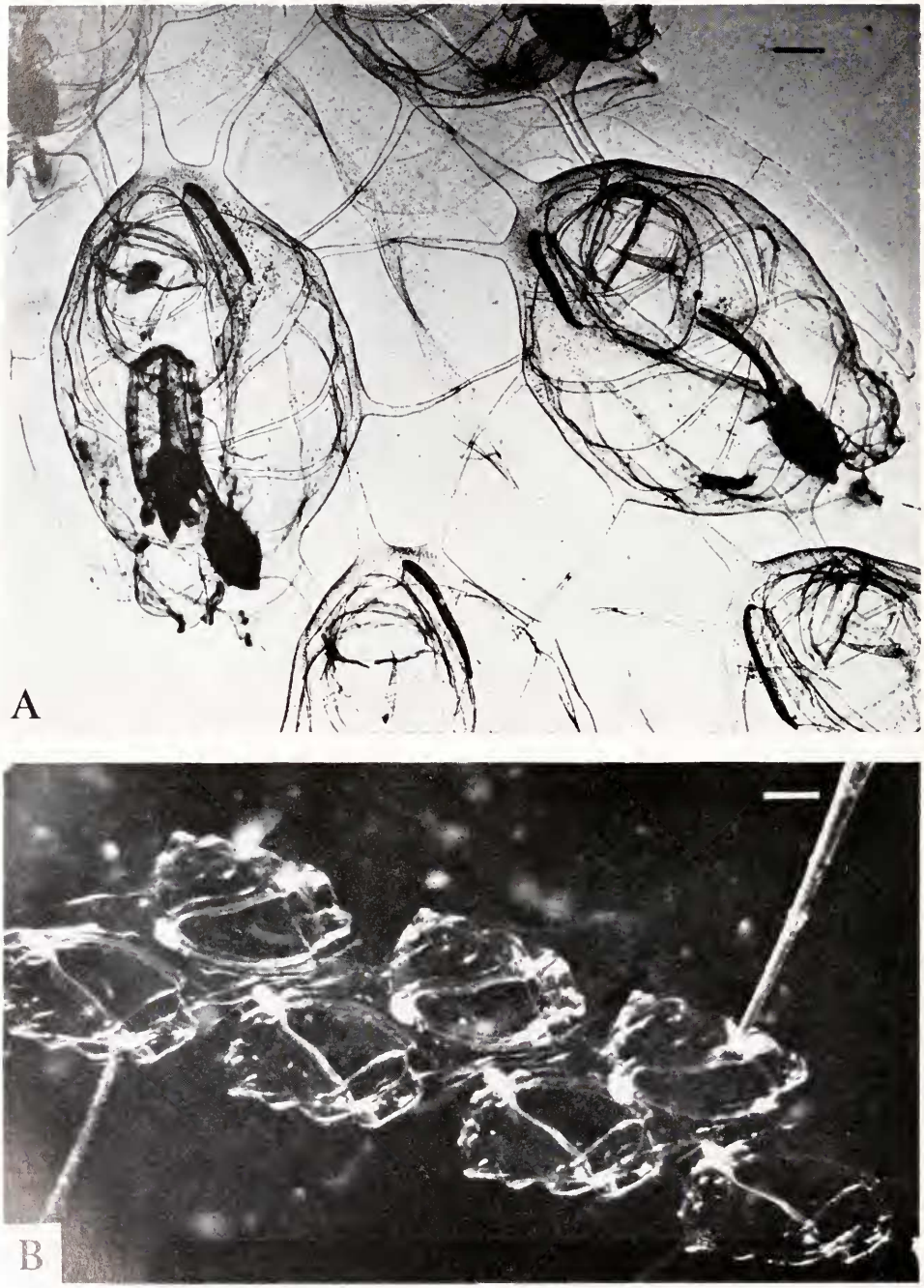

FIGURE 10. A. *Thalia democratica*, part of a chain of young blastozooids showing interconnections (photo by Claude Carré). Note oozoid embryo in the individual on the left

be strategically placed for sensing movement of adjacent zooids might function in locomotory coordination (Bone, 1959). Coordination could theoretically be achieved either by conduction of impulses between individuals or, mechanically, by tension or compression changes picked up by sense organs or excitable epithelia in each zooid in turn and relayed along the colony.

Electrical recordings have been made from intact salp chains restrained within a stockade of pins, or held by suction tubes (Fig. 10B). If a zooid in any part of the chain is stimulated so as to evoke OSPs, OSPs appear successively in other individuals along the chain (Fig. 11). In *Pegea* the transmitted response which followed a single shock was initially a burst of two or three OSPs rather than a single OSP, but after several stimuli, single OSPs were seen. The OSP burst may be similar in different zooids but, just as often, it varies either in the number of frequency relationships of the pulses. This argues against a simple through-conducting epithelial pathway, which should show a one for one relationship between stimuli and SPs and, judging by the behavior of SP systems within single zooids, should be less prone to fatigue and threshold changes than we find to be the case in chains.

By contrast, in stolons of *S. fusiformis,* OSPs are conducted in a simple one to one fashion with single electrical stimuli without obvious fatigue or threshold changes and with a short refractory period (18 msec).

There is some indication that "conduction velocity" (strictly speaking, the rate at which OSPs appear successively down the chain) changes as the chain matures. In a stolon, SPs were through-conducted at 5.25 cm/sec; in a very young chain grown from a stolon in the lab, the rate was 9.0 cm/sec; in slightly older chains retrieved from the plankton, 12.5 cm/sec. Mature chains of *Pegea* conducted OSPs at 5.25 cm/sec.

On the basis of present evidence, it appears probable that stolons and young chains possess epithelial through-conduction pathways which are later lost or modified. The spread of OSPs in older chains might represent sequential generation of these events in response to a signal mediated either by nerves or mechanically.

What, if any, causal relationship exists between the spread of skin pulses along the chain and the locomotory changes which also spread along the chain has not been determined, and until more is known about the precise role of the skin pulse systems at the level of the individual zooid, this question cannot be usefully pursued.

Discussion

This study has confirmed the main points in Fedele's account of locomotory organization in salps; namely, the timing of the firing sequence of the valve and body muscles as the basis for forward and reverse swimming, the neurogenic origin of the swimming rhythm, and the coordinated behavior of salp chains. We have shown further that the body wall muscles do not propagate but show graded respon-

(scale is 1 mm). B. *Salpa fusiformis,* chain of young blastozooids with recording electrodes attached to the test. A salp so attached receives little stimulation from the recording electrodes and behaves much as in nature (scale is 1 cm).

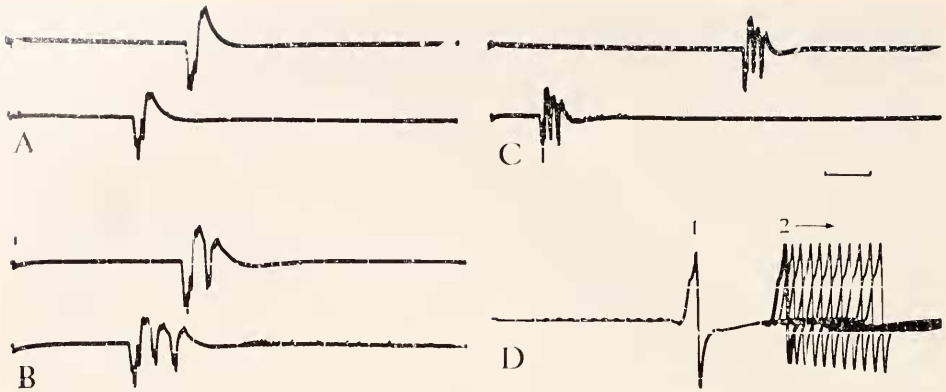

FIGURE 11. Recordings of outer skin pulses from salp chains (A-C, *Pegea confoederata*, D, stolon of *Salpa fusiformis*): A and B are recordings from two zooids separated by another zooid, simple (A) or complex (B) responses follow single shocks (scale is 200 msec); C. recording from two zooids with 20 zooids in between (scale is 500 msec); and D. two shocks were delivered every 10 secs to the stolon with the interval between the shocks being reduced by 5 msec at each sweep until the abosolute refractory period was reached sweeps are superimopsed, so that all first responss (1) coincide, while second responses (2 onwards) are spread out in time, (scale is 200 msec).

sivity, and we have recorded from a pacemaker cell and from presumed locomotory motoneurons in the brain. The brain resembles several other invertebrate ganglia in being able to generate a complex motor program without the need for peripheral input or feedback (Kater, Heyer and Kaneko, 1975).

It would be of considerable interest to clarify the mechanism whereby the switch from foward to reverse locomotion is achieved. The brain is capable of delaying the output to one end of the animal by a considerable interval (40 msec in the case of forward swimming). The photic responses of salps also deserve further study. The simple ON and OFF responses noted here could be mediated by much less complex photoreceptors than salps are known to possess and probably represent only a fragment of the total picture. Salp photoreceptors show hyperpolarizing receptor potentials as do those of vertebrates (Gorman, McReynolds and Barnes, 1971).

The major new feature of salp behavior to come to light in our work is the ocurrence of conducting epithelia. There are at least four independently conducting skin pulse systems. Their territories are distinct, as are their electrical signals. In other tunicates with conducting epithelia there is unequivocal evidence that skin pulses affect locomotory activity; each time a skin pulse is evoked, a change in locomotion follows. In the tadpole larva of one ascidian (*Dendrodoa*) SPs inhibit swimming (Mackie and Bone, 1976); in the larvacean *Oikopleura* they initiate or accelerate locomotion (Bone and Mackie, 1975). In the latter, uncoupling the skin from the central nervous system surgically or by drugs is the only way of preventing SPs from affecting locomotor activity.

In *Oikopleura* skin pulses enter the CNS through a pair of neurosensory receptors located in the skin. These receptors have bristle-like processes and can function as mechanoreceptors in the absence of skin pulses, affecting locomotion

in the same way (Bone and Mackie, 1975, confirmed by recent, unpublished observations). The inhalent and exhalent valves of salps (*S. maxima*) are equipped with sensory receptors having long processes which reach to the surface of the test (Bone, 1959), and similar receptors occur elsewhere in the outer skin. On the larvacean model, these receptors would serve both as mechanoreceptors and as entry routes for skin pulses. Fedele (1933c) states that receptors are present in the inner lining of the pharynx, but we have not observed nerve endings in the gill or pharyngeal epithelia, where ISPs are propagated. Nerves may, however, be associated with the endostyle and peripharyngeal bands (Fedele, 1933a), and receptors in these regions might serve as ISP entry points.

In salps the pathway from skin to CNS seems to be more labile than in tadpoles and larvaceans, for skin pulses sometimes have no effect on locomotion. The salp system is certainly more complex than that of the smaller tunicates in that the locomotory response to skin stimulation can vary depending on where the stimulus is applied. ASPs evidently enter the nervous system anteriorly and cause locomotory arrest or reversal. OSPs are conducted over the entire outer epithelium and might therefore enter the nervous system at several different levels in succession, the order in which the respective neurosensory units were excited being a critical factor in determining the locomotory response. This however is entirely speculative. In the case of ISPs, there is no firm evidence regarding their function, and the situation is complicated here by uncertainty about the distribution of sensory endings.

Taking into account the work on larvaceans and ascidian tadpoles, it now appears that conducting epithelia are widely present in free-swimming tunicates, but to date there has been no satisfactory evidence for their existence in sessile ascidians. Of all the tunicates, the salps probably offer the best opportunities for analysis of the interactions beween conducting epithelia and nerves, and we hope to pursue our investigations in this direction.

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Summary

1. Various observations by M. Fedele on the mechanism of forward and reverse locomotion, on the neurogenic origin of the locomotor rhythm and on the coordinated behavior of salp chains are confirmed or extended. *Salpa fusiformis* was the species chiefly studied.

2. The striated muscle fibers of the body wall exhibit nonpropagative, graded responsivity. The fibers are multiply-innervated. Adjacent fibers are not electrically coupled.

3. Intracellular recordings are reported from a pacemaker and presumed motor neurons in the brain. The locomotor rhythm is exhibited by deafferented and isolated brains. In the intact animal, sensory input can modify the rhythm and alter the firing sequence of the muscles. The rhythm is accelerated by reduction, and inhibited by elevation of the ambient light intensity.

4. The outer skin is a conducting epithelium. The cells conduct action potentials at *ca.* 17 cm/sec and are connected by gap junctions. Three other independently conducting inner epithelial territories are described. Propagated impulses in the excitable epithelia are believed to enter the nervous system *via* neurosensory processes in the skin, extending the effective fields of these receptors.

5. Salp chains show coordinated responses but, except in their earliest developmental stages, impulses are probably not through-conducted along the chain, but are relayed from one zooid to the next by an unknown mechanism.

6. Comparisons are drawn between salps and other pelagic tunicates where conducting epithelia have previously been reported.

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